DNA GENEMAPPER ID-X ANALYSIS OF RAW DATA

A. SCOPE

A.1 GeneMapper *ID-X* analysis software is used to analyze the raw data collected by the 3XXX genetic analyzer:

A.1.1 A spectral file is applied to the raw data to create a single baseline as well as to correct for spectral overlap and produce peaks of the individual colors.

A.1.2 A size curve is created using co-injected GS 500 LIZ (YFiler), GS 600 LIZ (GlobalFiler) labeled fragments of known size and the unknown peaks are assigned a size by interpolation.

B. QUALITY CONTROL

Not applicable

C. SAFETY

Not applicable

D. REAGENTS, STANDARDS AND CONTROLS

Not applicable

E. EQUIPMENT & SUPPLIES

E.1 Computer
E.2 GeneMapper ID-X software

F. PROCEDURES

F.1 Open the GeneMapper *ID-X* program with a blank project window or from the GeneMapper *ID-X* program by selecting **FILE>NEW PROJECT** or select the  from the task bar.

F.2 From the project window select **EDIT>ADD SAMPLES TO PROJECT** or select the  from the task bar.

F.3 Navigate to the collected data saved on the I: drive, highlight the necessary files and click **ADD TO LIST**. Click **ADD** to import the file or **CLEAR** if these are not the samples to be imported.

F.4 Select 3XXX Data Analysis or other table setting from the table setting drop down list located in the task bar. To create a new table setting, see Document 1813.

F.5 Select the analysis method by clicking the first empty cell in the Analysis Method column in the samples view. Select the desired analysis method from the drop down list. To create a new analysis method, see Document 1812. Click the **ANALYSIS METHOD** column header to select the entire column. Select **EDIT>FILL DOWN (CTRL + D)** to apply the selected analysis method to all samples.

F.6 Click on the first empty cell in the **Panel** column and open the AmpFLSTR_Panels_v1X (for YFiler), ormGlobalFiler_ v1 (for GlobalFiler). Double click on the YFiler_v1x, or GlobalFiler_Panel_v1 from the drop down list. Use the **EDIT>FILL DOWN** feature to place the panel in each sample row.

F.7 Click on the first empty cell in the Size Standard column and select CE_G5_HID_GS500 (YFiler), or GS600_LIZ_(60-460) from the drop down list. Use the **EDIT>FILL DOWN** feature to place the size standard in each row. Make sure that the 250 bp peak is not included in the ILS 600 size standard table.

F.8 In the **Sample Type** column select the type of sample from the drop down menu. **ALLELIC LADDER** must be selected for ladders. **POSITIVE CONTROL** may be selected for positive controls and **NEGATIVE CONTROL** may be selected for negative controls. If choosing the same sample type, double click on the box and it will fill it in automatically.

F.9 Click the green arrow analyze button found on the task bar. A **Samples Not Normalized** dialog box will open; select "Don't show again until the next session" and OK. The **SAVE PROJECT** dialog box will open. Save the project. The GeneMapper *ID-X* project does not need to be saved permanently since the raw data has been saved already.

F.10 The analysis summary window will appear once all of the data has been analyzed. It organizes samples into ladders, controls and samples. It further sorts all files into all thresholds met and one or more thresholds not met.

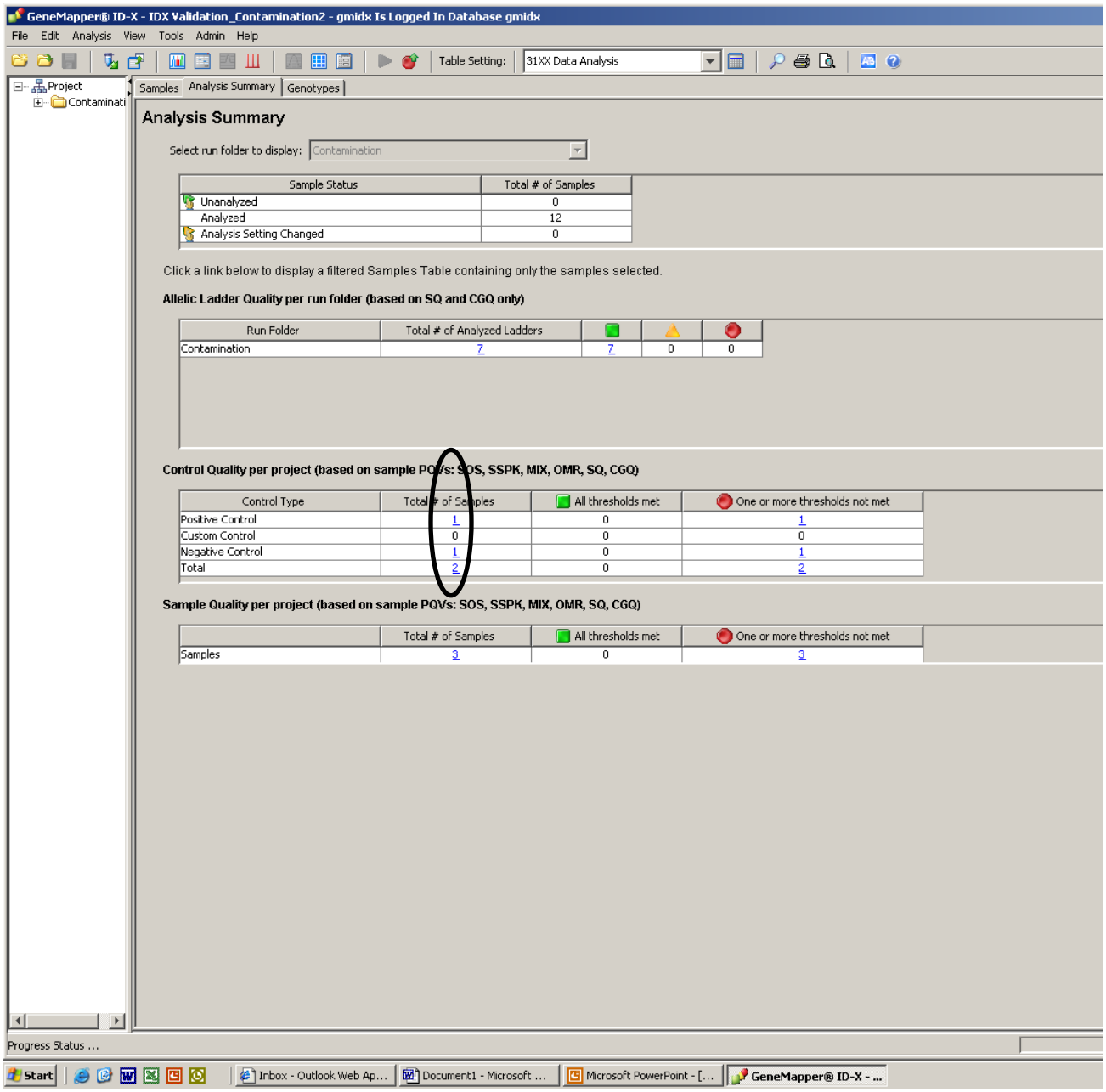

Figure 1

F.11 The samples can be accessed by clicking on the blue link within each box (Figure 1).

F.12 In the samples tab, a peak detection algorithm includes a sizing quality (SQ) value to assess the sizing of a sample. Using the Size Match Editor can change this value, however this value is not to be changed.

F.12.1 The flags in the SQ column assess sizing quality.

F.12.2 Observe that all flags in the SQ column are green squares, indicating that all samples passed sizing criteria.

F.12.3 If a red octagon appears the sample was not sized

F.13 GeneMapper *ID-X* v 1.5 & 1.6 may be used as a partial expert system, and therefore, ladders found in the **all analysis requirements met** column (green square) do not need manual review by the analyst.

F.13.1 If the analyst wants to manually check the ladders, click on the link within each sample column. They can also be accessed through the **SAMPLES** tab in the **analysis summary window** (Figure 1).

F.13.2 To manually check **ALLELIC LADDERS**: View the electropherogram by clicking the **DISPLAY PLOTS** button in the task bar. Select a plot setting from the **PLOT SETTINGS** drop down list. Verify that the allelic ladder is called correctly for each marker.

F.14 Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **DISPLAY PLOTS** button or add using the task bar. There are several options available to view the electropherogram, or one may be created (See Document 1814).

F.15 Edit any labels as appropriate i.e. spike, background, or stutter by right clicking on the allele and select **RENAME ALLELE LABEL>CUSTOM ARTIFACT LABEL**. Type the name in the **CUSTOM ARTIFACT LABEL** dialog box and click ok. Use existing labels by clicking on desired label. The custom allele label may be saved to a predefined list for later use by clicking the **ADD TO PREDEFINED LIST** box at the bottom of the **CUSTOM ARTIFACT LABEL** dialog box.

F.16 Review the remaining sample files. Evaluate the following parameters:

F.16.1 Peak shape and height (optimal values are typically between 75-6,000 RFU for 3130 instrument data, and above 100 RFU with an average heterozygous peak height of between 2758 – 4832 RFU for the 3500xL CE platform).

F.16.2 Spectral quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks).

F.16.3 Peak profile (examine for artifact peaks, i.e. spikes)

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 GeneMapper ID-X Software Reference Guide
H.2 GeneMapper ID-X Software Getting Started Guide
H.3 GeneMapper ID-X – Technical Note Peak Morphology
H.4 GeneMapper ID-X - User Bulletin Bin Overlap
H.5 GeneMapper ID-X v1.6 - Technical Note Peak Height Detection
H.6 GeneMapper ID-X v1.6 - User Bulletin - New Features A